Part 18

Gender Identity

Sexual Differentiation of the Human Brain in Relation to Gender-Identity, Sexual Orientation, and Neuropsychiatric Disorders

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Abbreviation	
AR	Androgen receptor
BSTc	Central nucleus of the human bed nucleus of the stria terminalis
DES	Diethylstilbestrol
CAIS	Complete androgen insensitivity syndrome
CAH	Congenital adrenal hyperplasia
ER	Estrogen receptor
FtM	Female-to-male transsexual person
InM	Intermediate nucleus
INAH	Interstitial nucleus of the anterior hypothalamus
MtF	Male-to-female transsexual person
SCN	Suprachiasmatic nucleus
SDN-POA	Sexually dimorphic nucleus of the preoptic area
SON	Supraoptic nucleus

Brief History

The topic of sexual differentiation of the brain has from the start raised violent reactions in our society. When, in the early 1970s, I (DFS) gave my first university lectures on sexual differentiation of the brain, the broadly accepted view on the importance of the social environment was put into words by Money et al. (1975):

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"Gender identity is sufficiently incompletely differentiated at birth as to permit successful assignment of a genetic male as a girl. Gender identity then differentiates in keeping with the experience of rearing." This idea also offered guidelines for feminist thinking: all the differences between the sexes as far as behavior, profession, and interests were concerned were thought to be forced upon women by the male-dominated society. The world famous feminist Germaine Greer stated in her book "The Female Eunuch" (1970): "It is known for example that sex hormones do enter the brain, but no correlation between that physiological fact and mental capacity has ever been established, although it has been assumed," thus denying the existence of the studies of Phoenix et al. (1959) and many others. During those first university lectures, I became aware of the first row of the lecture hall, filled with female medical students busy knitting and crocheting. It was transparent that they did not want to hear what I was discussing nor my views of it. When I switched off the lights to show my slides, there was a loud protest: they couldn't see their needlework! From that moment on, I decided to show my slides spread out over the entire lecture, with very dim lighting throughout. The ladies of the front row sent a delegation to the Rector to insist on a nonchauvinist lecturer. Apparently such a person was not available: the matter never came up again.

When we first reported a sex difference in the hypothalamus of man (Swaab and Fliers 1985), we heard some disapproving noises from the feminist movement because a sexual difference in the brain did not fit their philosophy that every difference in behavior, and therefore in the brain, had been forced upon women by society. In an interview in the Dutch magazine HP (January 17, 1987), biologist Joke't Hart responded to our publications with: "If I were to accept that there are sex differences in such fundamental aspects as the structure of the brain, I might as well stop being a feminist." I have never heard from her since that interview. Since then, many observations have been done that call into question the idea of a dominant importance of the environment on our differentiation into men and women. We have discussed a few of these observations below.

After our report of the first difference in the brain between homosexual and heterosexual men (see Swaab and Hofman 1990), the response was unexpectedly massive and negative. It all began in December 1988, with a monthly that nobody reads, the Academy News (the organ of the Royal Netherlands Academy of Arts and Sciences KNAW). In it, researchers employed by institutes of the KNAW are interviewed about their current research, so I told them something about our studies of sexual orientation and gender identity. This story was picked up by a good Dutch reporter, Hans van Maanen, who wrote two scientifically sound articles that for some reason went on to cause a riot you wouldn't believe. After all these years, the exact reason for this violent emotional and completely wrongly directed massive reaction is still not entirely clear to me: apparently the taboo of the biological background of our sexual orientation was very strong, at a time when everything was considered makeable. There was a group of homosexual men who almost religiously believed that all men were gay but that only some had the courage to come out of the closet. They called being gay a political choice. My response was that I did not see what was political about it and that the choice of your sexual

orientation is made for you, in the womb. There was great anger, and in 3 weeks' time, many hundreds of articles appeared in the press. The COC (the Dutch lesbian, gay, bisexual, and transgender organization) was "amazed" when they learned of the research. Professor Rob Tielman was one of my fiercest antagonists at the time. He publicly vilified the research by calling it "in bad taste," which set the tone, and said that I should have first asked him for permission to research and publish, which of course was utter nonsense. Later on, he backpedaled in an interview and said: "In the field of gay studies I am closest to Swaab" and "I am one of those people who tend to take the biological component very seriously." But in the mean time, the editor of the Gay Krant (the gay newspaper), Henk Krol, had also given his two cents' worth: "This kind of research feeds the idea that homosexuality is a disease. This supports once again the discrimination of gays."

There were questions in Parliament about my research by Peter Lankhorst, a member of the PPR party. His questions ended on my desk, via the desks of the Minister and the President of the KNAW, and my answers took the same route back. We endured phone terror at home, day and night, and I received threats per post, addressed "To the SS-doctor Dr. Mengele-Swaab" that informed me, often in ungrammatical sentences full of spelling mistakes: "Nazi, seen you on the TV. Villain's face. We homophiles will kill you. As example the Spiritual leader Khomeini-Iran about the Englishman." I did not take these threats very seriously and commented that if their killing talents matched their writing talents I did not run much of a risk. I would probably feel differently today. I also received mail that said: "You probably would have liked to be able to work under Mengele in Auschwitz."

Committees reviewed my work, and security measures were taken for my lecture in the Medical Center of Amsterdam. There were bomb scares at my institute (that I didn't take seriously either), my children were harassed about the matter at school, and one Sunday morning, I awoke to a demonstration right outside my door, an occurrence that the late Gerard Reve (famous Dutch gay author) wrote about in his inimitable way in his compilation of 1995 entitled: *Sunday morning without worries*. He wrote:

> Only then Professor Swaab's serious omission became clear: he had neglected to ask for permission for his research from the gay union, the C.O.C. Well, the consequences showed up and made themselves heard: on a Sunday morning a large group of motivated individuals appeared at Professor Swaab's house in Amstelveen, chanting all together and loudly: 'Dick, cut into your own pr(..)!' Most peculiar, when you think that, although Professor Swaab was investigating sexuality, he studied the brain, and not the sexual organs. However, as the followers of this union do not have brains, but do possess sexual organs, it did make sense in a way.

It took 3 weeks for the ruckus to die down. Ayatollah Khomeini pronounced a fatwa against Salman Rushdie because of his book *The Satanic Verses*, and instantly the entire focus shifted to the British-Indian author. When the dust around my "affair" had settled and I had remained standing, the president of the KNAW, David de Wied, gave an interview to the Telegraaf newspaper, in which he supported me and said that such an affair should never happen again.

Fig. 102.1 Cartoon by Peter van Straaten after announcement of our finding that the hypothalamic suprachiasmatic nucleus was twice as large in homosexual men than in heterosexual men (Swaab and Hofman 1990). "Wim has also such a large hypothalamus, isn't it?" (With permission by Kajsa Blomberg (agent van Peter van Straaten))

But there were nice reactions too, such as Peter van Straaten's cartoon (Fig. 102.1) and some personal ads in the weekly "Vrij Nederland," such as the one that said: "Nice guy (37, 1.87, 87 kg, blonde and blue) with large hypothalamus seeks. . . ." and "LARGE suprachiasmatic nucleus, p.o. box 654 Wageningen." However, it took 17 years before the Gay Krant changed its view of that period and published an article entitled: "Angry gays got it all wrong." It was also remarkable that after all that time, Rob Tielman, in his column, was unable to get beyond a slightly caustic: "Stubborn Swaab."

When we then published the first sex reversal in the transsexual brain with my first Chinese Ph.D. student as the first author (Zhou et al. 1995), we only received positive reactions. Transsexuals pounced upon this paper to enforce a change of sex in their birth certificate or passport in countries where that had not been possible. The paper was also used in the European Court of Justice for this purpose and played a role in bringing about legislation about this issue in England.

When my coauthor Dr. Ai-Min Bao, a student of Dr. Zhou and me, entered 10 years later the field of sex differences in the stress-coping systems in relation to depression, no emotional reactions were heard anymore. This in spite of the fact of the two times higher prevalence of major depression in women was not so long ago

generally considered to be due to the suppressive male society. The field of sexual differentiation of the human brain seems thus, after a violent adolescence, to have finally entered adulthood.

Introduction

Sex differences in the brain are, e.g., reflected in gender identity (the conviction of belonging to the male or female gender), sexual orientation (hetero-, homo-, bisexuality, or pedophilia), and in the risk for neuropsychiatric disorders. The fetal brain develops into the male direction during the intrauterine period by a direct action of testosterone on the developing nerve cells or in the female direction by the absence of a testosterone surge.

From the first days after birth, sex differences are already expressed in human behavior. For example, female neonates prefer to look at human faces, while male infants look more at the mechanical mobiles on their first days of life, respectively. In childhood, girls prefer to play with dolls, while boys prefer toy cars and balls, which is already obvious at 3–8 months of age. Such toy preferences cannot be explained by an effect of social pressure, since when toys for boys and girls were offered to green vervet monkeys, the female ones consistently chose the dolls and would even show anogenital sniffing, while the male ones were more interested in playing with the toy cars and balls (Fig. 102.2).

Such sex differences in behaviors seem to be crucially dependent on the effect of testosterone on the fetus since girls who were exposed to high testosterone levels in the womb, in case of congenital adrenal hyperplasia (CAH), tended to choose boys as playmates, playing preferentially with boys' toys and exhibited some male-typical personality features. It is thus logical to propose that the sex differences in playing behavior seem to originate early in our evolution, before the hominids, and are imprinted during our intrauterine development under the influence of testosterone. A similar sex difference is seen in the children's spontaneous drawings. A Japanese study showed that 5–6 years old girls tend to draw human figures, flowers, and butterflies in bright colors, while boys, on the other hand, prefer to draw more technical objects, soldiers, weapons and fighting, and means of transport in birds'-eye view compositions and in darker colors (Fig. 102.3). Girls who had CAH showed male drawing characteristics, even if the CAH was treated immediately after birth (Fig. 102.4). Apparentely fetal exposure to higher levels of male hormones has lasting effects on behavior and artistic expression. It should be noted that atypical children's toy preferences are not necessarily predicting a gender identity disorder in adulthood. Rather, it is predictive of homosexuality (see below).

Sex differences are found in adult behaviors as well. In men, aggressive behavior has also been related to prenatal testosterone levels. More importantly, sex differences are also reflected in the prevalence of contracting neuropsychiatric disorders such as depression, anxiety, schizophrenia, drug abuse, and Alzheimer's disease (AD) (Table 102.1).

Fig. 102.2 Examples of a female and a male animal contacting toys. The female animal (*left*) appears to be conducting an anogenital inspection of the toy doll, similar to inspections of infant vervet monkeys. The male animal (*right*) appears to be moving the car along the ground in a manner similar to that a child might use (From Alexander and Hines 2002, Fig. 2, © Elsevier)

Organizational Effects of Sex Hormones During Early Development

The fetal gonads develop under the influence of a cascade of genes, starting with the sex-determining gene on the Y chromosome (*SRY*). The production of testosterone and the peripheral conversion of testosterone into dihydrotestosterone between weeks 6 and 12 of pregnancy are essential for the formation of a boy's penis, prostate, and scrotum, while the development of the female sexual organs in the womb is primarily based upon the absence of fetal androgens. Once the differentiation of these sexual organs is settled, sexual differentiation of the brain happens, mainly under the *organizing effects* of sex hormones on the brain that are permanent. Later, during puberty, the brain circuits that have been organized in the womb will be *activated* by sex hormones.

There are two critical periods in human development where testosterone levels are known to be higher in boys: the first period occurs during midpregnancy, while the second takes place in the first 3 months after birth. These fetal rising and neonatal rising of testosterone, together with the functional steroid receptor activity, are thought to fix to a major degree the development of structures and circuits in the brain for the rest of a boy's life and are called "programming" or "organizing" effects. The "activating" effect of rising hormone levels during puberty stimulates

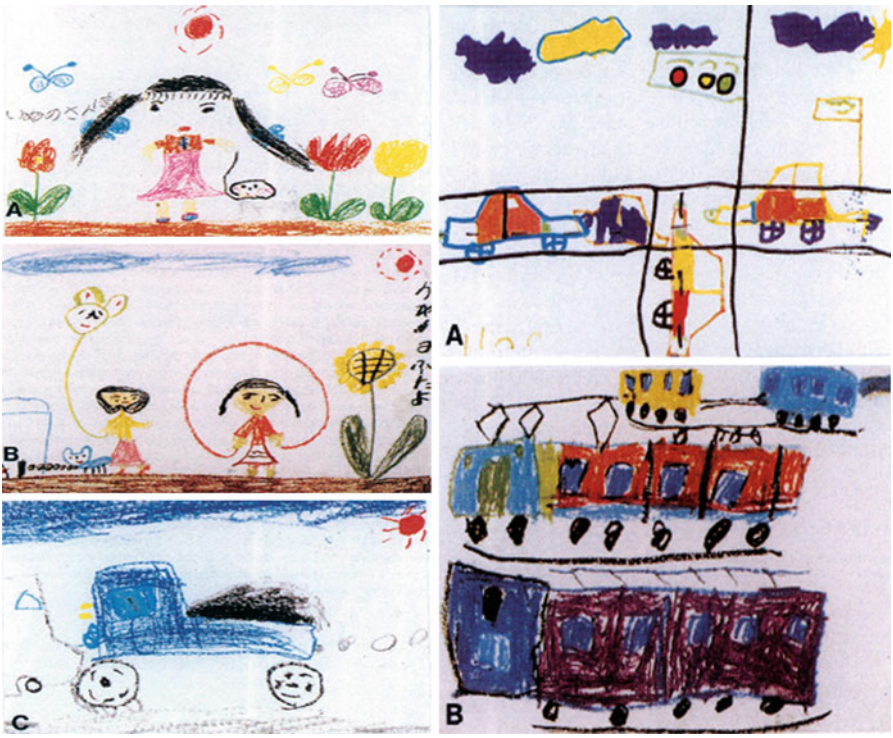

Fig. 102.3 *Left*: (**a**) picture drawn by a 5-year-old girl; (**b**) picture drawn by a 6-year-old girl; (**c**) picture drawn by a 5-year-old boy. *Right*: (**a**) bird's-eye view of an intersection drawn by 6-year-old boy. (**b**) Trains are drawn in a pile by a 5-year-old boy (From Iijima et al. (2001), Fig. 3, © Elsevier)

circuits and behavioral patterns that have been set up during the development in a masculinized and defeminized direction for male brains or in a feminized and demasculinized direction for female brains.

As sexual differentiation of the genitals takes places much earlier in development (i.e., in the first 2 months of pregnancy) than sexual differentiation of the brain (which starts in the second half of pregnancy and becomes overt upon reaching adulthood), these two processes may be influenced independently. In rare cases, this may result in transsexuality, i.e., people with male sexual organs who feel a female identity, or vice versa. It also means that in the event of an ambiguous sex at birth, the degree of masculinization of the genitals may not always reflect the degree of masculinization of the brain. Structural differences in the brain resulting from the interaction among genes, sex hormones, and developing brain cells are thought to be the basis of sex differences in a wide spectrum of behaviors including gender role (behaving as a man or a woman in society), gender identity (the conviction of belonging to the male or female gender), and sexual orientation (heterosexuality, homosexuality, or bisexuality).

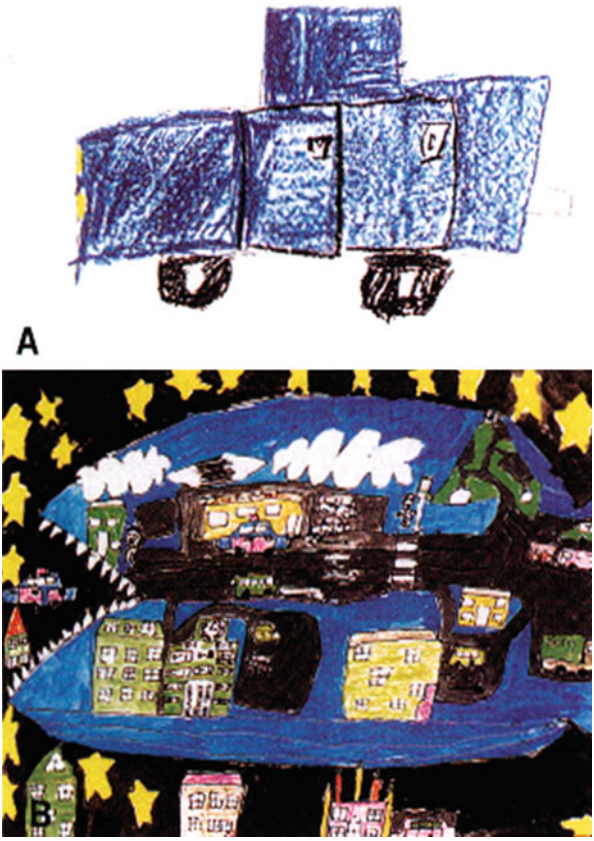

Fig. 102.4 (**a**) A car drawn by a 5-year-old girl with congenital adrenal hyperplasia (CAH). (**b**) Bird's-eye view picture drawn by a 7-year-old girl with CAH (From Iijima et al. (2001), Fig. 4, © Elsevier)

Sex Differences in the Human Brain in Relation to Gender Identity and Sexual Orientation

A sex difference in brain weight in allometric relation to body weight is already present from the age of 2 years. Sex differences in adult brain structures have been observed from the macroscopic to the ultramicroscopic levels, together with a large number of functional sex differences in different brain regions, only a few of which could be mentioned in this chapter. In the hypothalamus, our group first found a structural sex difference in the sexually dimorphic nucleus of the preoptic area (SDN-POA), which was later also called the interstitial nucleus of the anterior hypothalamus-1 (INAH1) or the intermediate nucleus (InM) of the human hypothalamus. The SDN-POA was found to be 2.5 times larger and to contain 2.2 times more cells in men than in women (Fig. 102.5). Such sex differences develop only after the age of 5 and disappear temporarily after the age of 50 (Fig. 102.6).

Table 102.1 Ratios for women over men suffering from a selection of neurological and psychiatric diseases (for references, see Swaab (2003))

Disease	Women:men (%)
Rett syndrome	100:0
Postoperative hyponatremic encephalopathy with permanent damage or death	96:4
Anorexia nervosa	93:7
Lymphocytic hypophysitis	90:10
True (central) precocious puberty	90:10
Hypnic headache syndrome	84:16
Bulimia	75:25
Senile dementia of the Alzheimer type	74:26
Multiple sclerosis	67:33
Anxiety disorder	67:33
Anencephaly	67:33
Post-traumatic stress disorders	70:30
Dementia	64:36
Unipolar depression, dysthymia	63:37
Whiplash	60:40
Severe learning disability	38:62
Substance abuse	34:66
Amyotrophic lateral sclerosis	33:67
Stuttering	29:71
Schizophrenia	27:73
REM sleep behavioral disorder	24:76
Male-to-female vs. female-to-male transsexuals	28:72
Dyslexia	23:77
ADHD	20:80
Autism	20:80
Sleep apnea	18:82
Kallmann syndrome	17:83
REM sleep disorder	13:87
Gilles de la Tourette syndrome	10:90
Kleine-Levin syndrome	0:100

Abbreviations: *REM* rapid eye movement, *ADHD* attention-deficit/hyperactivity disorder

Two other cell groups, INAH-2 and INAH-3, which showed larger volumes in men compared to women (2.8 and 2 times greater, respectively) were described by Allen et al. Recently, our group has confirmed the sex difference in volume and neuron number of the INAH3 and an absence of such a difference in the INAH4 (Fig. 102.7), which fully agreed with previous data. Other structural sex differences have been found in, e.g., the subcortical human anterior commissure, the interthalamic adhesion, the corpora mamillaria, and the cortex.

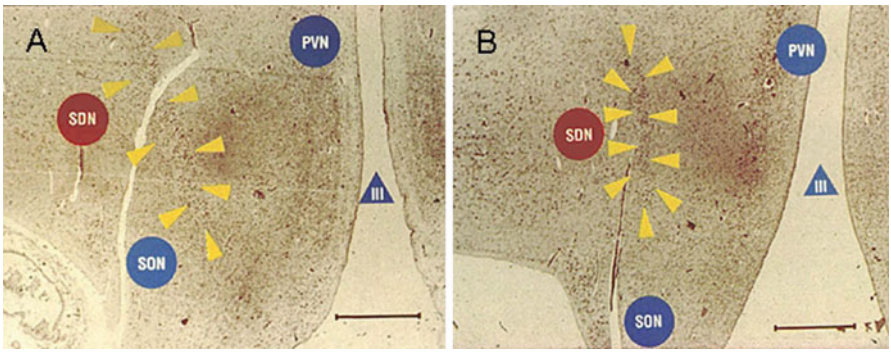

Fig. 102.5 Thionine-stained frontal section (6 μm) of the hypothalamus of (**a**) a 28-year-old man and (**b**) a 10-year-old girl. Arrows show the extent of the sexually dimorphic nucleus of the preoptic area (SDN-POA = INAH-1 = ImN). Note that the male SDN is larger than that of the female. Bar represents 1 mm (From Bao and Swaab 2010, Fig. 4, © Sage)

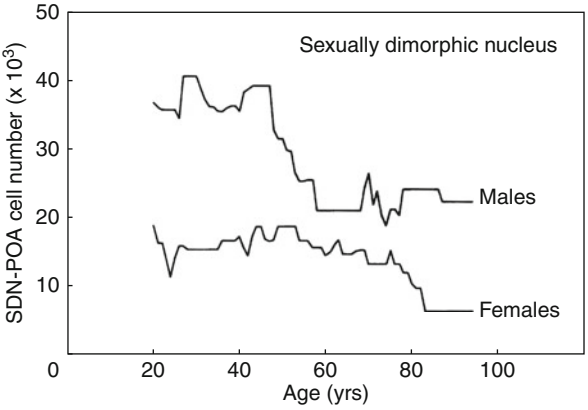

Fig. 102.6 Age-related changes in the total cell number of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in human hypothalamus. Brains from 30 human subjects (13 males and 17 females), ranging in age from 10 to 93 years of age, without a neurological or psychiatric disorder, were obtained by autopsy. Volume and cell density measurements were made on serial hypothalamic sections (6 μm) and were used to calculate the total number of cells. The general trend in the data is enhanced by smoothing the data points using polynomial regression and a scatter plot smoothing procedure with an equally weighed moving average. Note that in males, SDN cell number steeply declines between the ages of 50 and 70 years, whereas in females, a more gradual cell loss is observed around the age of 80 years. These curves demonstrate that the reduction in cell number in the human SDN in the course of aging is a nonlinear, sex-dependent process (From Hofman and Swaab 1989, Fig. 5, © Wiley)

Programmed Gender Identity Is Irreversible

During the 1960–1970s, it was generally thought that a child is born as a tabula rasa and is subsequently forced into the male or female direction by the society conventions. For example, John Money stated (1975) that, "Gender identity is sufficiently incompletely differentiated at birth as to permit successful assignment of a genetic male as a girl. Gender identity then differentiates in keeping with the experiences of rearing." He also believed that gender imprinting does not start until the age of 1 year and that its development is well advanced by the age of 3–4 years. His view had devastating consequences in the case of David Reimer, i.e., the John-Joan-John case, in which an 8-month-old boy, who lost his penis due to a mistake during a minor surgery (i.e., correcting a phimosis), was made into a girl based upon Money's opinion. The child's testicles were removed before he reached the age of 17 months in order to facilitate feminization; in addition, he was dressed in girl's clothes, received psychological counseling by Money, and was given estrogens in puberty. According to Money, this child developed as a normal female. However, it became later clear that this had not been the case at all. Reimer never identified as female, and he began to live again as male at age 14. Unfortunately, due to years of severe depression, financial instability, and a dissolving marriage, Reimer committed suicide in 2004. This story illustrates the strength of the irreversible programming influence during the intrauterine period on gender identity. Other cases describing the results of enzymatic disorders or of cloacal exstrophy also support the existence of early permanent programming of gender identity in the brain by biological factors such as intrauterine androgen exposure, rather than by social environment and learning.

Atypical Brain Structures in Transsexuality

The most extreme gender-identity disorder is transsexuality. It consists of the unshakeable conviction of belonging to the opposite gender, which tends eventually to lead to a request for hormonal treatment and sex-reassignment surgery. There is no indication that postnatal social factors could be responsible for this disorder. On the other hand, only 23% of childhood gender problem cases will lead to transsexuality in adulthood. A number of factors have been found to be risk factors for transsexuality (see Table 102.2), including chromosomal abnormalities and polymorphisms of the genes for the estrogen receptor (ER)α and ERβ, androgen receptor (AR), and aromatase or cytochrome P450 (CYP)-17. Abnormal hormone levels during early development may also play a role, as girls with CAH have an increased chance of becoming transsexual. It should be noted that although the likelihood of transsexuality developing in the CAH cases is 100–300 times higher than normal (in population 1:10,000–1:30,000), the risk for transsexuality in CAH is still only 1–3%, whereas the probability of serious gender problems in this group is 5.2%. The consensus is, therefore, that girls with CAH should be raised as girls, even if their genitals are masculinized. Epileptic women who were given phenobarbital or

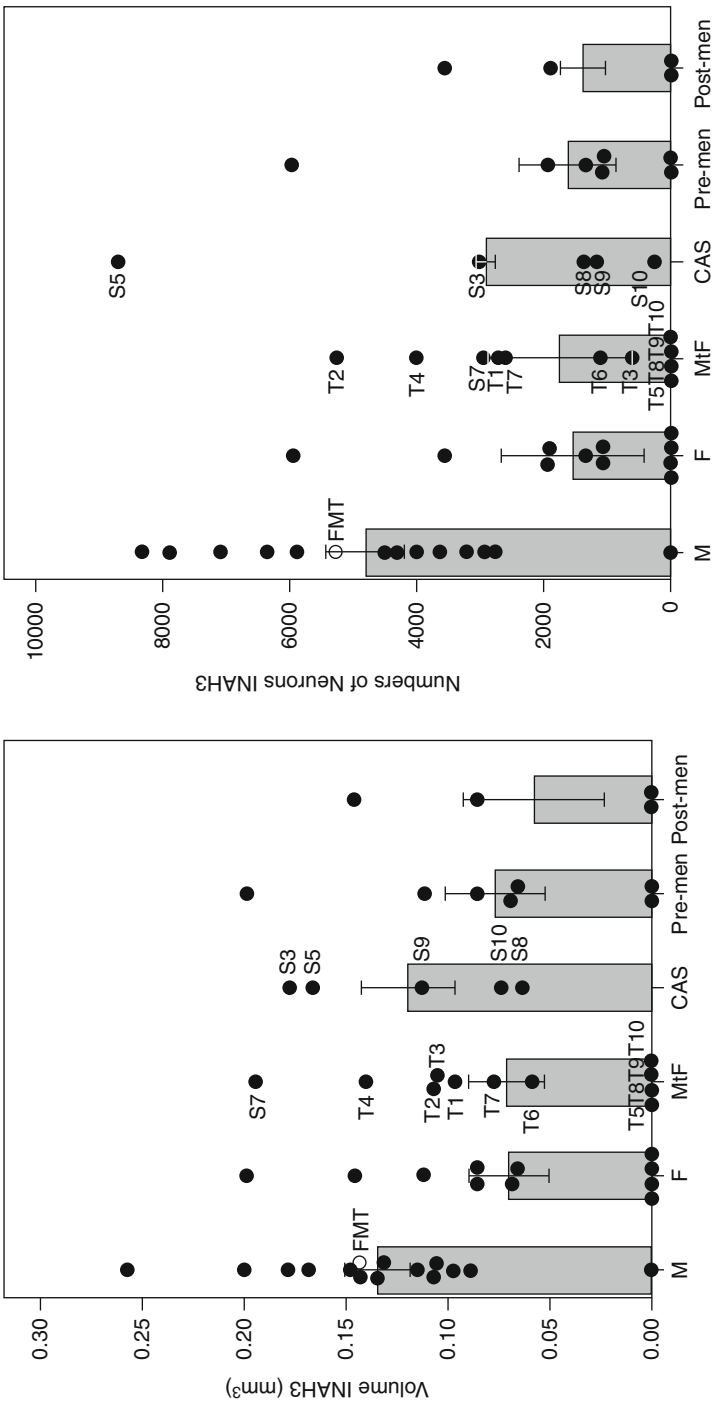

Fig. 102.7 (**a**) The interstitial nucleus of the anterior hypothalamus (INAH)-3 volume in thionine staining in different groups, according to their gender identity and hormonal changes in adulthood. *M* control male group, *F* control female group, *MtF* male-to-female transsexual group (the individuals are designated as T with a number), *CAS* castrated male group (the individuals are designated as S with a number), *Premen* premenopausal women, *Postmen*: postmenopausal women. *Bars* represent means and standard errors of the mean (SEM). MtF and F groups were statistically different compared to the M group ($P < 0.018$ and $P < 0.013$, respectively). Hormonal changes in adulthood (CAS vs. M and Premen vs. Postmen groups) showed no difference in INAH3

Table 102.2 Prenatal factors that influence gender identity that may result in transsexuality (for references, see Swaab and Garcia-Falgueras (2009))

Genetic factors
Rare chromosomal disorders
Twin studies
Polymorphisms in ERβ, androgen receptor, and aromatase genes
Hormones
Phenobarbital/diphantoine taken by pregnant mother
Cloacal exstrophy
5 α-Reductase-2 or 17β-hydroxy-steroid-dehydrogenase-3 deficiency
Girls with CAH
Complete androgen insensitivity syndrome results in XY heterosexual females with feminine identity
Immunological factors?
Fraternal birth order effect
Social factors?
Postnatally no evidence

Abbreviations: *CAH* congenital adrenal hyperplasia, *DES* diethylstilbestrol

diphantoine during pregnancy also have an increased risk of giving birth to a transsexual child since both of the chemicals may change the metabolism of the sex hormones and can act on the sexual differentiation of the child's brain. Furthermore, homosexual male-to-female transsexual people were found to have a later birth order and more brothers than sisters, suggesting the presence of immunological processes during pregnancy, directed toward products of the Y chromosome.

The theory of the origins of transsexuality is based on the fact that the differentiation of sexual organs appears before the sexual differentiation of the brain. As the two processes are not synchronous, it could be that they take different routes under the influence of differently timed factors. If this is the case, one might expect to find, in transsexuals, female sexual organs with a male brain, vice versa. Indeed, such reversals have been found by us in the central nucleus of the human bed nucleus of the stria terminalis (BSTc) and in the INAH3, two brain structures that are also involved in sexual behavior in rodents.

Fig. 102.7 (continued) volume. Note that the volume of the female-to-male transsexual subject (FMT, in the M group, 51 years old) is in the male range. Note also that in a considerable proportion of the females (F) and MtF individuals, the INAH-3 is small or absent. A gender dysphoric male-to-female patient who was not treated in any way (S7, put in the MtF group, 84 years old) showed a male value for INAH3 volume. (**b**) Distribution of the INAH3 number of neurons among different groups. Statistically differences were found between men (M) and women (F) ($P < 0.029$) and between men (M) and MtF transsexual groups ($P < 0.002$). The female-to-male transsexual subject (FMT, in the M group, 51 years old) had a masculine INAH3 number of neurons, while the gender dysphoric nontreated patient (S7, put in the MtF group, 84 years old) had a similar number of neurons to the other transsexuals examined (From Garcia-Falgueras and Swaab 2008, Figs. 5 and 6, © Oxford Journals)

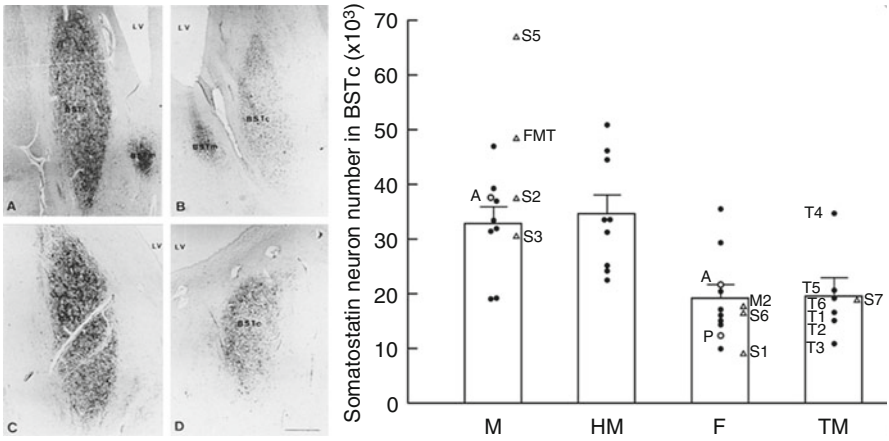

Fig. 102.8 *Left*: representative sections of the central nucleus of the bed nucleus of the stria terminalis (BSTc) innervated by vasoactive intestinal polypeptide (VIP). (**a**) heterosexual man; (**b**) heterosexual woman; (**c**) homosexual man; (**d**) male-to-female transsexual. Scale bar, 0.5 mm. *LV* lateral ventricle. Note that there are two parts of the BST in A and B: small medial subdivision (BSTm) and large oval-sized central subdivision (BSTc) Note also the sex difference (**a** vs. **b**) and the fact that the male-to-female transsexual (**d**) has a female BSTc in size and type of innervation (From Zhou et al. (1995), Fig. 2, © The Endocrine Society). *Right*: distribution of the BSTc neuron numbers among the different groups according to sex, sexual orientation, and gender identity. *M* heterosexual male reference group, *HM* homosexual male group, *F* female group, *TM* male-to-female transsexuals. The sex hormone disorder patients S1-6 and M2 indicate that changes in sex hormone levels in adulthood do not change the neuron numbers of the BSTc. The difference between the M and the TM group ($p < 0.04$) becomes also statistically significant according to the sequential Bonferroni method if S2, S3, and S5 are included in the M group or if S7 is included in the TM group ($p \leq 0.01$). Note that the number of neurons of the female-to-male transsexual (FMT) is fully in the male range. Whether the transsexuals were male oriented (T1, T6), female oriented (T2, T3, T5), or both (T4) did not have any relationship with the neuron number of the BSTc. The same holds true for heterosexual and homosexual men. This shows that the BSTc number of somatostatin neurons is not related to sexual orientation. A: AIDS patient. The BSTc number of neurons in the heterosexual man and woman with AIDS remained well within the corresponding reference group, so AIDS did not seem to affect the somatostatin neuron numbers in the BSTc. P: Postmenopausal woman. S1 (♀ 46 year of age): adrenal cortex tumor for more than 1 year, causing high cortisol, androstenedione, and testosterone levels. S2 (♀ 31 year of age): feminizing adrenal tumor that induced high blood levels of estrogens. S3 (♂ 67 year of age): prostate carcinoma; orchiectomy 3 months before death. S5 (♂ 86 year of age): prostate carcinoma, prostatectomy, orchiectomy, and antiandrogen treatment for the last 2 year. S6 (♀ 25 year of age): Turner syndrome (45,X0; ovarian hypoplasia). M2 (♀ 73 year of age): postmenopausal status (From Zhou et al. (1995), Fig. 1, © The Endocrine Society)

In men, the BSTc volume was twice as large as in women and contained twice as many of somatostatin neurons (Fig. 102.8). The same was true for the INAH3, which was found to be 1.9 times larger in men than in women and contained 2.3 times more neurons (Fig. 102.7). In addition, a female INAH3 and BSTc have been found in MtF transsexual persons (Figs. 102.6, 102.7). Moreover, in the only female-to-male (FtM) transsexual person available for our studies so far, the

BSTc and INAH3 had all the male characteristics. Furthermore, a functional imaging study also found that MtF transsexuals had sex-atypical hypothalamus activation by pheromones. These observations thus support the neurobiological theory about the origin of transsexuality, i.e., it is the sizes, the neuron numbers, the functions, and connectivity of brain structures, and not the sex of their sexual organs, birth certificates, or passports that match their gender identities. Unfortunately, the sex difference in the BSTc volume does not become apparent until early adulthood, which means that this nucleus cannot be used for early diagnosis of transsexualism.

Factors Influencing Sexual Orientation

Sexual orientation refers to the gender (male or female) to which a person is attracted, i.e., to the opposite sex (heterosexual), to the same sex (homosexual), and to both sexes (bisexual). Sexual orientation is also determined during early development, under the influence of genetic background and factors that influence the interactions between sex hormones and the developing brain and awakened during puberty under the influence of sex hormones. The apparent impossibility of changing a people's sexual orientation is a major argument against the role of the society or environment in the emergence of homosexuality, as well as against the idea that homosexuality is a *lifestyle choice*.

Twin and family studies have indicated over 50% of genetic component in the development of sexual orientation. However, it is unclear which genes exactly play such a role. A number of genetic studies have suggested a maternal transmission, i.e., an X-linked inheritance. The X chromosome has accumulated genes involved in sex, reproduction, and cognition. A meta-analysis of four linkage studies suggested that Xq28 may play an important role in male homosexuality. However, 18 years after the initial findings, the exact genes involved have not yet been identified. A different technique also indicated that women with gay sons had an extreme skewing of X inactivation, closely associated with gene silencing by DNA and/or histone methylation, as compared to mothers without gay sons. Although this unusual methylation pattern supports a possible role of the X chromosome in male homosexuality, its mechanism of action is far from clear. Given the complexity of the development of sexual orientation, it is likely to involve many genes. A genome-wide linkage screening indeed identified several chromosomal regions and candidate genes for further exploration.

Several additional factors during development influence our sexual orientation. An abnormal hormone level is apparent from the large percentage of bisexual and homosexual girls with CAH (Table 102.3). An estrogen-like substance, diethylstilbestrol (DES), was once prescribed to some two million pregnant women in the USA and Europe between 1939 and 1960 for the purpose of preventing miscarriage. It turned out, however, that DES did not prevent miscarriage, while a small dosage of it not only gives a slightly elevated risk of cervical cancer but also increases the chance of bisexuality or homosexuality in girls. The chance that a boy will be

Table 102.3 Prenatal factors that may influence sexual orientation (for references, see Swaab and Garcia-Falgueras (2009))

Genetic factors
Twin studies
Molecular genetics
Hormones
Girls with CAH
DES
Chemical factors
Prenatal exposure to nicotine, amphetamines, or thyroid medication
Immune response?
Fraternal birth order effect
Social factors
Stress in the mother during pregnancy
Being raised by transsexual or homosexual parents does not affect sexual orientation

Abbreviations: *CAH* congenital adrenal hyperplasia, *DES* diethylstilbestrol

homosexual increases with the number of his older brothers is known as the fraternal birth order effect. It is putatively explained by the progressive immunization of some mothers to Y-linked minor histocompatibility antigens by each succeeding male fetus. Prenatal exposure to nicotine, amphetamine, or thyroid-gland hormones increases the chances of giving birth to lesbian daughters, while a stressed pregnant woman has a greater chance of giving birth to a homosexual son. There lacks solid proof that postnatal development may play any important role in directing sexual orientation. On the contrary, children who were born after artificial insemination with donor sperm and were raised by lesbian couples were heterosexually oriented. There also lack proofs for the idea that homosexuality is the result of a deficient upbringing or that it is a lifestyle choice or an effect of social learning. Therefore, it is to our opinion a total irrational opinion that some people still forbid their children to play with homosexual friends mainly because of a sort of fear that homosexuality is contagious or can be learned.

Brain Structures in Relation to Sexual Orientation

Several structural and functional differences in the brain have been described to be potentially related to sexual orientation. Our group found the first difference in the suprachiasmatic nucleus (SCN), the biological clock, which was twice as large in homosexual as in heterosexual men. In 1991, LeVay reported that homosexual men have a smaller volume of INAH-3. Allen and Gorski reported homosexual men have, compared with heterosexual men, larger anterior commissure, a structure that is usually larger in women than in men, which takes care of the left-right temporal cortex connection and may thus be involved in sex differences in cognitive abilities and language. Further Savic and Lindström have found that the difference in

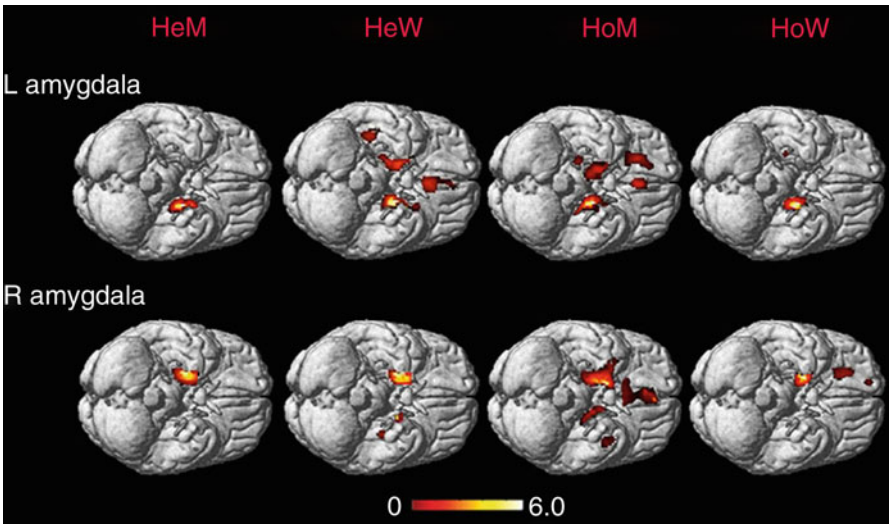

Fig. 102.9 Covariations with the respective amygdala seed region in hetero- and homosexual subjects. The Sokoloff scale indicates T values. Clusters detected at T = 3.0 are superimposed on the standard magnetic resonance image of the brain. In homosexual men (HoM), like in heterosexual women (HeW), the connections were more widespread from the *left* amygdale, in homosexual women (HoW) and heterosexual men (HeM), on the other hand, from the *right* amygdala. Furthermore, in HoM and HeW, the connections were primarily displayed with the contralateral amygdale and the anterior cingulate, in HeM and HoW with the caudate, putamen, and the prefrontal cortex (From Savic and Lindstrom 2008, Fig. 1, © The National Academy of Sciences of the United States of America)

anterior commissure size may possibly be related to the sex-atypical hemispheric asymmetries observed in homosexual men and women. No difference, however, was found in the size or number of neurons in the BSTc in relation to sexual orientation (Fig. 102.8).

Functional brain scanning also revealed differences in relation to sexual orientation: the hypothalamus of homosexual men was not as responsive to fluoxetine as that of heterosexual men, indicating different activities of the serotoninergic system. It is a well-known phenomenon that unconscious personal communication is carried on through pheromones, Using PET, Savic et al. explored the influence of pheromones excreted in perspiration in concentrations 10 times higher in men than in women on sexual behavior. They found such pheromones stimulated the hypothalamus of heterosexual women and homosexual men in the same way, but heterosexual men were not stimulated by a male scent, which suggests that pheromones may contribute to determining our partner choice. In a follow-up study, they found that lesbian women, as compared to heterosexual women, reacted in a sex-atypical, almost reciprocal, way to pheromones. This group, using respective magnetic resonance volumetry and PET measurements, also found sex-atypical cerebral asymmetry and functional connections in homosexual subjects that cannot be primarily ascribed to learned effects but suggest a linkage to neurobiological entities (Fig. 102.9).

Brain Changes in Pedophilia

As a topic of taboo until recently, little is known about the risk factors for pedophilia. The familial transmission of pedophilia indicates genetic involvement. Compared to homosexual- and heterosexual-control subjects, pedophiles showed decreased gray matter volumes in the ventral striatum, extending into the nucleus accumbens, the orbitofrontal cortex, and the cerebellum. In addition, smaller volumes of hypothalamus, amygdala, septal regions, substantia innominata, and BST were observed in pedophiles. Sexual interest in children was found to be associated with lower white matter volumes of the superior fronto-occipital fasciculus. Moreover, central processing of visual sexual stimuli seems to activate more strongly subcortical regions involved in reward processing signals in homosexual pedophiles compared to homosexual nonpedophiles. In a comparable study, heterosexual pedophiles were found to show an activation of dorsolateral prefrontal cortex instead of the normal orbitofrontal cortex stimulation. These data indicate an atypical brain development in pedophiles, leading to atypical brain structures and processing in relation to sexual behavior.

Neurobiological Mechanisms of Sexual Differentiation of the Brain

The main mechanism responsible for gender identity and sexual orientation in human involves a direct effect of testosterone on the developing brain, as shown in different disorders. Complete androgen insensitivity syndrome (CAIS) is caused by different mutations in the gene for AR. The affected XY males develop as phenotypical women and experience "heterosexual" orientation and fantasies without gender problems. On the other hand, when a male fetus has a deficiency of 5α-reductase-2 or 17β-hydroxy-steroid dehydrogenase-3, preventing peripheral testosterone from being transformed into dihydrotestosterone, a "girl" will be born with a large clitoris. These XY children are generally raised as girls. However, when testosterone production increases during puberty, the "clitoris" grows into penis size and testicles descend, the children's build begins to masculinize and becomes muscular. Despite the fact that these children are initially raised as girls, the majority (60%) will change into heterosexual males, apparently by the *organizing* effect of testosterone on early brain development and by the *activating* effect of testosterone in puberty. Boys who are born with a cloacal exstrophy, i.e., with bladder exstrophy and partly or wholly absent penis, are usually changed into girls immediately after birth. A survey showed that in adulthood, only 65% of these children who were changed into girls continued to live as girls, and when individuals with gender dysphoria were excluded, the figure dropped to 47%. These examples make it clear that the direct effect of testosterone on the developing boys' brain and a lack of effect on the developing girls' brain are very crucial for the development of gender identity and sexual orientation.

It should be noted, however, that although sex hormones are very important for gender identity and sexual orientation, sexual differentiation of the brain is not

caused by hormones alone. Genes, too, play a key role in it, with *SRY* and *ZRY* as the possible candidates for this action since they are expressed up to very advanced ages in the human brain, even though strictly speaking, the role of these genes in sexual differentiation stops during development. In addition, it has been found that 50 genes are expressed at different levels in the brains of male and female mouse fetuses, even before the hormones come into play. Moreover, epigenetic changes such as the acetylation and methylation of multiple proteins recruited by sex hormone receptor function are important for the developing nervous system as they affect adult sex differences in rodent brain and behavior. Epigenetic changes are found in the germline – and are therefore inherited – and in somatic cells and generally persist only for one lifetime and are largely context dependent. The context may be variables such as steroid hormones or endocrine-disrupting chemicals, experiences as far-reaching as early child abuse, or events as mild as context-dependent learning. Recent evidence shows that ARs and ERs interact with histone-modifying enzymes, which are associated with neural sexual differentiation. Circulating testosterone activates AR and is converted into estrogen in the brain via aromatase. Extensive sexual dimorphism in the number and projections of aromatase-expressing neurons has been demonstrated, and the masculinization of these cells was found to occur independent of AR. However, it was possible to induce it in female rodents by either testosterone or estrogen, indicating a role for aromatase in sexual differentiation. It is suggested that aromatase or the aromatization of testosterone into estrogen is important in activating rat male-specific aggression and urine-marking behavior, i.e., the development and activation of neural circuits that control male territorial behaviors. The role of the aromatase mechanism, in addition to a direct effect of testosterone, on the developing human brain for gender identity and sexual orientation is, however, at present unknown.

Sex Differences in the Prevalence of Brain Disorders

Factors that interfere with the interactions between sex hormones and the developing brain systems in the womb may permanently influence not only later behavior but also the risk of neuropsychiatric disorders. The proportions of cases range from more than 75% women in Rett syndrome, lymphocytic hypophysitis, anorexia and bulimia nervosa, and hypnic headache syndrome to more than 75% men in dyslexia, attention-deficit/hyperactivity disorder, autism, sleep apnea, Gilles de la Tourette syndrome, rabies, Kallmann syndrome, and Kleine-Levin syndrome (Table 102.1). Sex differences are not only shown in the prevalence but also in the signs, symptoms, and the course of the disorders. Men not only suffer from schizophrenia 2.7 times more often than women, they are also prone to a more severe form, a poorer premorbid functioning experience, earlier onset, more negative symptoms, and cognitive defects and exhibit a greater number of structural brain abnormalities, such as a more severe enlargement of the lateral ventricles. In addition, relapses of male patients are more severe, and their response to neuroleptic medication is less favorable. Female patients, on the other hand, display more affective symptoms, auditory hallucinations, and

persecutory delusions. Moreover, an interaction with gender was observed in the second trimester of pregnancy when prenatal exposure to maternal stress was studied as a risk factor for schizophrenia, with male fetuses held higher risk ratio. Factors that produce normal sexual dimorphism in the brain, particularly in the cortex, may be associated with modulating insults producing schizophrenia.

Other examples of a sex difference in a neurological disease are those following restricted left-hemisphere lesions, resulting in aphasia in 41% of the males and 11% of the women, whereas manual apraxia was found in 6% of the women and 42% of the men. After severe subarachnoid hemorrhage, mortality in women was lower (37%) than in men (53%), while the incidence of favorable outcome was higher in women (42%) than in men (26%). Female traumatic brain injury patients also had a better predicted outcome than male patients. According to some studies, the prevalence of AD is higher in women than in men, although some other researchers did not find an association between AD and gender and suggested that the excess number of female AD is only due to the longer life expectancy of women. However, the observation of an increased number of nucleus basalis of Meynert neurons containing more AD pathology in women compared to age-matched men and the association found between AD and a locus on the X chromosome support the presence of sex differences in AD. Lower endogenous estradiol levels are correlated with poor cognitive, behavioral, and functional status in older but nondemented women, while higher free testosterone levels are associated with better scores on visual and verbal memory, visuospatial functioning, and visuomotor scanning in elderly nondemented men. Several studies have reinforced the idea that the postmenopausal decrease in estrogen levels may be an important factor in triggering the pathogenesis of AD since women with high serum concentrations of bioavailable estradiol are less likely to develop cognitive impairment than women with low concentrations. In old men, endogenous testosterone levels are not associated with a risk for cognitive decline and AD, whereas higher estrogen levels increase such a risk.

Whether sex differences in the brain that arise in development (*organizing effects*) are indeed the basis for the sex difference in neurological or psychiatric diseases has still to be established. Alternative mechanisms involved may be the immediate effects of differences in circulating sex hormone levels (*activating effects*), caused by sex hormone-stimulated gene transcription, as we have shown in depression (see below).

Sex Differences in Stress-Coping Behavior

Stress as a psychological and biological term was firstly coined in the 1930s by Selye. It refers to the consequences of the failure of an organism – human or animal – to respond appropriately to emotional or physical threats, whether actual or imagined. Once we sense stressors, our bodies' defenses kick into high gear in a rapid, automatic process known as the "fight-or-flight" reaction or the stress response.

All human behavior is evolutionarily designed to maintain, via stress coping of everyday life, the continuance of the gene pool and ensure the survival of human beings. Both animal and human studies showed the female brain has a different innate strategy to handle stress from that of the male brain. Young human males are more prone than females to take risks in relation to conflicts, outdoor activities, and car driving. In addition, men are more likely to show physical aggression; they commit 89% of all murders and 99% of all sexual crimes, while women show more indirect aggression such as spreading vicious rumors about the target person, gossiping behind this person's back, telling others not to associate with the intended victim, or even making up stories about that person. Research also indicates that sex differences with respect to indirect aggression are present among children as young as 8 years old and increase through age 15, and they seem to persist into adulthood.

Sex and Age Differences in the Hypothalamo-Pituitary-Adrenal (HPA) Axis

The HPA axis is the key system in the regulation of the stress responses. In brief, the hypothalamus releases corticotropin-releasing hormone (CRH) in response to a stressor, which triggers the pituitary gland to secret adrenocorticotropin (ACTH) into the bloodstream and subsequently causes corticosteroid releasing from the adrenal cortex (mainly cortisol in humans). Cortisol as a major stress hormone also acts on many other organs and brain circuits such as the hippocampus, amygdala, and prefrontal cortex, which also participate in the feedback regulation. Cortisol also exerts a negative feedback effect on the pituitary and hypothalamus to shut down the stress response after the threat has passed, together with neurotransmitters such as gamma-aminobutyric acid. Part of the CRH neurons in the hypothalamic PVN coexpress arginine vasopressin (AVP). When released together into the portal capillaries, AVP strongly potentiates the ACTH-releasing activity. In addition, circulating AVP from the supraoptic nucleus (SON) may induce ACTH release from the pituitary.

Sex differences in stress regulation have important implications for understanding physiological differences in the male and female brain and their impact on vulnerability in disorders associated with stress. Women are more expressive of emotions, tend to score higher on scales related to emotional experiences such as neuroticism, and have increased risk of suffering from depression and most anxiety disorders. Morphometric studies have shown sexual dimorphism in several brain structures implicated in emotional processing, such as the cingulate and ventrolateral prefrontal cortices (larger in women) and the medial temporal structures, including the amygdala (larger in men). These structural differences are hypothesized to be programmed by sex steroids early in development.

The HPA axis and autonomic responses tend to be lower in women between puberty and menopause compared to men of same age. Roca et al. have found that young to middle-aged (18–45 years) men showed increased stimulated ACTH and cortisol to either pharmacological (CRH) or physical (exercise) stressors, compared

with age-matched women. This result was obtained in the absence of sex differences in estradiol or testosterone levels since at the time of testing, the subjects underwent gonadal suppression with leuprolide acetate. In addition, the secretion of cortisol after exercise and the initial secretion (0–30 min) of ACTH to either the stressors are significantly larger in this group of men than women. This shows that the sex differences exist even in the absence of the characteristic differences in reproductive steroids. It has also been found that elderly men activate the HPA axis to a greater extent than women in response to psychological stress. Cortisol production rate is clearly higher in men than in women. Our group has also found gender differences in the number of CRH-expressing neurons in the human hypothalamic PVN, namely. (1) there is a significant age-related increase of CRH neurons in men, but not in women, and (2) men have significantly more CRH neurons than women. An abnormal hormone status, i.e., by castration or ovariectomy or by a sex-hormone-producing tumor, was accompanied by changes in CRH neuron number. Age-related activation of CRH neurons could be affected by a series of factors, such as a decreased function of the hippocampus, which is more sensitive to the process of aging than the PVN and which suppresses the activity of the HPA axis. In this respect, it is of interest that a sex difference has been reported in hippocampal aging, e.g., a significant age-related decline of hippocampal volume was found in men, but not in women. Increasing insensitivity of the feedback of cortisol on the HPA axis may be another factor involved in the activation of the HPA axis in men during aging. AVP is also involved in the stress response. Baseline AVP was found to be significantly higher in the elderly than in young men and women. In addition, men have higher AVP levels than women. Moreover, the posterior lobe of the pituitary is larger in boys than in girls. These sex differences agree with the higher metabolic activity found in AVP neurons in the SON in young men, as compared to women.

The Basis of the Sex Differences in Prevalence of Depression

The HPA axis is considered to be the "final common pathway" for a major part of the depressive symptomatology. Sex differences involving the regulation of the activity of the HPA axis take part in depression. Unipolar depression and dysthymia are twice as common in women as in men during the females' reproductive years. This can, at least partly, be ascribed to the close functional interaction between the HPA and the hypothalamo-pituitary-gonadal (HPG) axes and the fluctuation of circulating sex hormones in women, especially during menstruation, pregnancy, after giving birth, and during the transition of menopause. Lower testosterone levels were also found in severely depressed or dysthymic male patients. Men who have low total testosterone levels and a shorter CAG codon repeat length in the AR have a greater likelihood of becoming depressed, while supraphysiological doses of testosterone increased ratings of manic symptoms in men.

The higher prevalence of depression during female's reproductive ages with changes of hormones levels suggests that *fluctuations* of sex hormone levels play

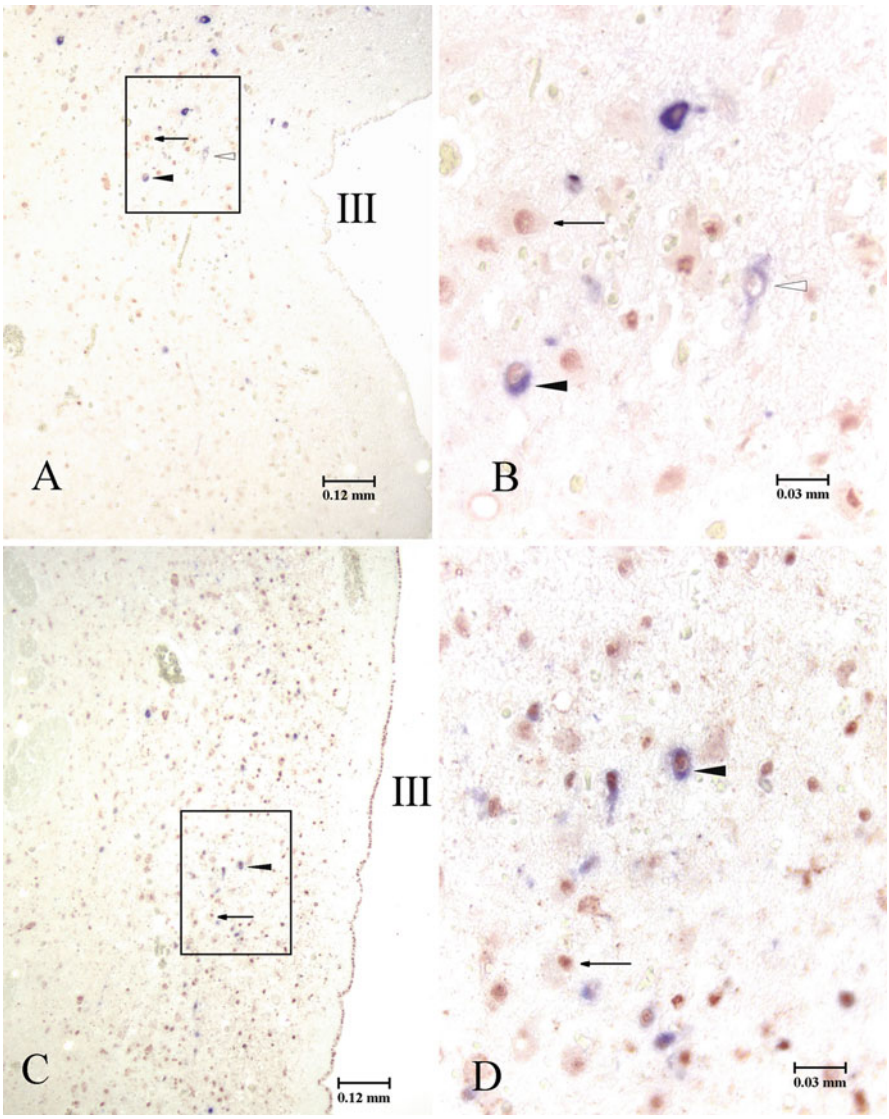

Fig. 102.10 Frontal section of the PVN in a control (C12) (**a**, **b**) and a patient with mood disorder (D10) (**c**, **d**) stained for CRH (*blue*) and ERα (*red*). (**b**) and (**d**) represent a 4× higher magnification of (**a**) and (**c**). The *arrows*, *solid* and *hollow arrowheads* in (**a**, **b**) and (**c**, **d**), indicate the same place in the preparation to facilitate comparison. Both sections show the central part (midlevel) of the PVN and contain the largest number of stained neurons. It is clear by comparing (**a**) with (**c**) and (**b**) with (**d**) that the number of stained neurons is markedly increased in this mood disorder patient. III: the third ventricle. The *arrow* points to an ERα nuclear single-staining cell; the solid arrowhead points to a cytoplasmic CRH-ERα nuclear double-staining cell and the hollow arrowhead points to a CRH single-staining cell (From Bao et al. (2005), Fig. 2, © Oxford Journals)

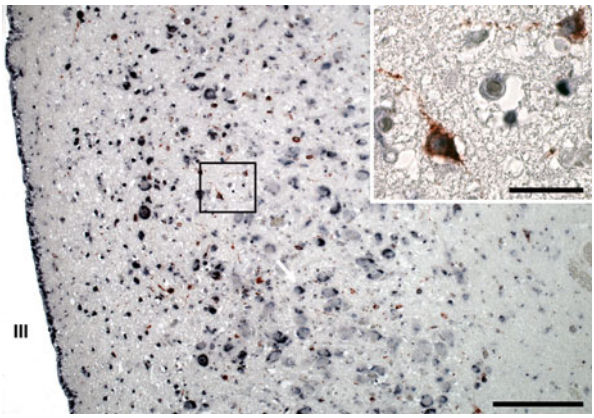

Fig. 102.11 Frontal section of the paraventricular nucleus (PVN) in subject (#00182) stained for corticotropin-releasing hormone (CRH) (*red*) and androgen receptor (AR) (*blue*). III: the third ventricle. The upper-right corner represents a higher magnification of the framed field and shows cytoplasmic CRH (*red*) and AR (*blue*) nuclear double-staining neurons. The *arrow* points to some AR single-staining cells. *Bar* in the upper-right corner = 16 μm; in the lower right corner = 100 μm (From Bao et al. (2006), Fig. 4, © NPG)

a more important role on the vulnerability for mood disorders than the *absolute basal levels*. In this respect, it is of interest to find that female major depression (MD) patients had indeed significantly higher amplitudes of diurnal estradiol rhythms than controls. In addition, significant differences in the brain stress response circuitry were observed in different phases of the menstrual cycle, which indicates that females have been endowed with a natural hormonal capacity to regulate the stress response in a way that differs from males.

CRH neurons have been found to colocalize with sex hormone receptors, indicating a direct effect of sex hormones on these neurons. Both the nuclear ERα and the nuclear AR are present in CRH-expressing neurons in the human hypothalamic PVN (Figs. 102.10, 102.11). In addition, a correlated upregulation of CRH and nuclear ERα was observed in mood disorders, both in males and females. It is known that the human CRH gene promoter contains five perfect, half-palindromic estrogen-responsive elements (EREs), while animal studies have shown that estrogens stimulate CRH production. Moreover, an androgen-responsive element (ARE) has been identified in the CRH gene promoter region, which initiates a repressing effect of AR on CRH expression, which is in agreement with an animal study showing that androgens inhibit CRH production. Recently significantly increased CRH-mRNA levels were found in the PVN of the depressed patients accompanied by a significantly increased expression of ERα-mRNA and significantly decreased expression of AR-mRNA, which not only supported the role of sex hormones, with clear sex differences, in depression but also raises the possibility that a disturbed balance among the factors affecting CRH activity may contribute to the activation of the HPA axis. The observations

that estrogens stimulate while androgen inhibits CRH transcription help to explain the sex differences seen in the prevalence of MD, a disorder in which the HPA axis is regarded as the final common pathway for the pathogenesis of depression is activated.

Outlook

During the intrauterine period, a testosterone surge masculinizes the fetal brain, whereas the absence of such a surge results in a feminine brain. As sexual differentiation of the brain takes place at a much later stage in development than sexual differentiation of the genitals, these two processes can be influenced independently of each other. Sex differences in cognition, gender identity (an individual's perception of their own sexual identity), sexual orientation (heterosexuality, homosexuality, or bisexuality), and the risks of developing neuropsychiatric disorders are programmed into our brain during early development. There is no evidence that one's postnatal social environment plays a crucial role in gender identity or sexual orientation. Examples were given of the relationships between structural and functional sex differences of various brain areas and the way they change along with any changes in the supply of sex hormones on the one hand and sex differences in behavior in health and disease on the other. It will be clear that sex differences play a role in many, if not all brain functions, behavior and neuropsychiatric disorders, although to a different degree. The field of sex differences of the human brain will produce the coming years an avalanche of new insights by integrating data on human behavior and other brain functions with data obtained by postmortem brain studies and structural, functional, and chemical brain imaging studies in health and disease.

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